THE PATENTS ACT, 1970

IT IS HEREBY CERTIFIED THAT, the annex is a true copy of Application and provisional specification filed on 28.08.2002 in respect of Patent Application No. 779/MUM/2002 of Lupin Ltd., 159, CST Road, Kalina, Santacruz (E), Mumbai – 400 098, State of Maharashtra, India, an Indian company.

This certificate is issued under the powers vested in me under Section 147 (1) of the Patents Act, 1970.

Dated this 09th day of October 2003

M.A. Haafeez.

(M.A. HAAFEEZ)
ASST. CONTROLLER OF PATENTS & DESIGNS

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

FORM 1

THE PATENTS ACT, 1970
(39 of 1970)

APPLICATION FOR GRANT OF A PATENT

[See sections 5 (2), 7, 54 and 135 and rule 33A]

1. We , a) LUPIN LTD., b) 159, CST Road, Kalina, Santacruz (E), Mumbai - 400 098, State of Maharashtra, India, c) an Indian company

2. hereby declare -

   (a) that we are in possession of an invention titled "HERBAL EXTRACT CONTAINING A MIXTURE OF SAPONINS OBTAINED FROM SAPINDUS TRIFOLIATUS HAVING ANTICONVULSANT ACTIVITY AND USEFUL IN THE PROPHYLACTIC TREATMENT OF MIGRAINE AND OTHER INDICATIONS"

   (b) that the Provisional specification relating to this invention is filed with this application.

   (c) that there is no lawful ground of objection to the grant of a patent to us.

3. further declare that the inventor(s) for the said invention are

   1. (a) ARORA,SUDERSHAN KUMAR

      (b) LUPIN LTD (RESEARCH PARK), 46A/ 47A, NANDE VILLAGE, TALUKA MULSHI, PUNE – 411 042, MAHARASHTRA,INDIA

      (c) AN INDIAN NATIONAL

   2. (a) SRIVASTAVA, VANDITA

      (b) LUPIN LTD (RESEARCH PARK), 46A/ 47A, NANDE VILLAGE, TALUKA MULSHI, PUNE – 411 042, MAHARASHTRA,INDIA

      (c) AN INDIAN NATIONAL

   3. (a) ADDEPALLI, VEERANJANEYULU

      (b) LUPIN LTD (RESEARCH PARK), 46A/ 47A, NANDE VILLAGE, TALUKA MULSHI, PUNE – 411 042, MAHARASHTRA,INDIA

      (c) AN INDIAN NATIONAL

ORIGINAL

779/MUM/2002
Dt : 28.8.02

779 | मुंबई MUM | 2002

28 AUG 2002

4. (a) NATESAN, SRIDHAR

(b) LUPIN LTD (RESEARCH PARK), 46A/ 47A, NANDE VILLAGE, TALUKA MULSHI, PUNE – 411 042, MAHARASHTRA,INDIA

(c) AN INDIAN NATIONAL

5. (a) GOEL, RAJAN

(b) LUPIN LTD (RESEARCH PARK), 46A/ 47A, NANDE VILLAGE, TALUKA MULSHI, PUNE – 411 042, MAHARASHTRA,INDIA

(c) AN INDIAN NATIONAL

4. I/We, claim the priority from the application(s) filed in convention countries, particulars of which are as follows : NONE.

5. I/We state that the said invention is an improvement in or modification of the invention, the particulars of which are as follows and of which I/we are the applicant/patentee:
NOT APPLICABLE.

6. I/We state that the application is divided out of my/our application, the particulars of which are given below and pray that this application deemed to have been filed on _____ under section 16 of the Act
NOT APPLICABLE.

7. That we are the assignee of the true and first inventors.

8. That our address for service in India is as follows :

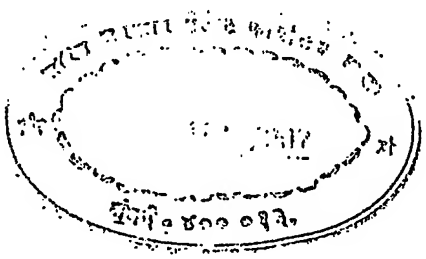
S. MAJUMDAR & CO., 5, Harish Mukherjee Road, Calcutta - 700 025, State of West Bengal. Phone : 0-33-4557484/4557485/4557486 ; Fax : 0-33-4557487/4557488.

9. We the true and first inventors for this invention declare that the applicant herein is our assignee.

1. (a) ARORA,SUDERSHAN KUMAR

(b) LUPIN LTD (RESEARCH PARK), 46A/ 47A, NANDE VILLAGE, TALUKA MULSHI, PUNE – 411 042, MAHARASHTRA,INDIA

(c) AN INDIAN NATIONAL



(ARORA,SUDERSHAN KUMAR)

2. (a) SRIVASTAVA, VANDITA

(b) LUPIN LTD (RESEARCH PARK), 46A/ 47A, NANDE VILLAGE, TALUKA MULSHI, PUNE – 411 042, MAHARASHTRA,INDIA

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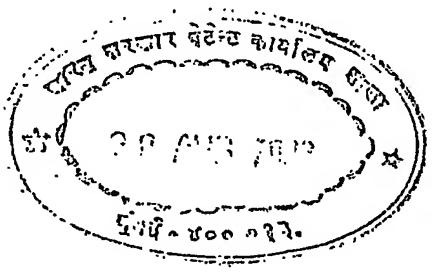
(c) AN INDIAN NATIONAL

GOEL, RAJAN

10. That to the best of our knowledge, information and belief the fact and matters stated herein are correct and that there is no lawful ground of objection to the grant of patent to us on this application.



11. Followings are the attachment with the application :

a) Provisional specification (in quadruplicate).
b) Statement and Undertaking on FORM-3 (in duplicate).
c) Fee of Rs. 5000/- In cheque No. 953068 dated 27th August 2002.

We request that a patent may be granted to us for the said invention.

Dated this 27th Day of August 2002.

S.Majumdar
Of S.Majumdar & Co.
Applicant's Agent

To
The Controller of Patents
The Patent Office
At Mumbai

FORM -2

THE PATENTS ACT, 1970
(39 OF 1970)

PROVISIONAL SPECIFICATION
(See Section 10)

1. TITLE OF INVENTION :
"HERBAL EXTRACT CONTAINING A MIXTURE OF SAPONINS OBTAINED FROM SAPINDUS TRIFOLIATUS HAVING ANTICONVULSANT ACTIVITY AND USEFUL IN THE PROPHYLACTIC TREATMENT OF MIGRAINE AND OTHER INDICATIONS"

ORIGINAL

2. a) LUPIN LTD., b) 159, CST Road, Kalina, Santacruz (E), Mumbai - 400 098, State of Maharashtra, India, c) an Indian company

The following specification describes the nature of the invention :

HERBAL EXTRACT CONTAINING A MIXTURE OF SAPONINS OBTAINED FROM *SAPINDUS TRIFOLIATUS* HAVING ANTICONVULSANT ACTIVITY AND USEFUL IN THE PROPHYLACTIC TREATMENT OF MIGRAINE AND OTHER INDICATIONS

FIELD OF THE INVENTION

The present invention relates to an aqueous herbal extract, containing a mixture of saponins prepared from the pericarp of *Sapindus trifoliatus*, exhibiting useful pharmacological activities, specially anticonvulsant activity in MES model, not known hitherto so far. The extract is specially suitable for the prophylactic treatment of migraine. The invention further relates to a process for preparation of the herbal extract, a pharmaceutical composition containing the said extract in combination with pharmaceutically acceptable carriers or vehicles. The invention also relates to a method of treatment of the aforesaid indications, specially the prophylactic treatment of migraine by administration of the pharmaceutical composition through intranasal route. The herbal extract of the present invention exhibits binding affinity for the receptor sites, which are known to be involved in anticonvulsant activity.

BACKGROUND OF THE INVENTION

Hemicrania, more popularly known as migraine nowadays, is a chronic episodic disorder characterized by attack of intense pulsatile and throbbing headache, typically unilateral in nature with or without aura. The symptoms associated with the attack are anorexia, nausea, vomiting and photo-and/or phonophobia. The pathophysiology of migraine is multifactorial and complex in nature [*Goodman and Gilman*, "Pharmacological Basis of Therapeutics", 9th Int. Ed., McGraw-Hill health professions Division, New York, **1996**, pp 486-89].

In addition to the above, several other theories/hypotheses have been proposed for explaining the clinical features of migraine, e g.

a. the vasodilation theory by J. Olesen et. al. in *Headache*, **1982**, 22, 242-48 and Lancet, **1981**, 2, 438-40, following detailed cerebral blood flow studies,

b. the trigeminovascular system pathway proposed by N. H. Raskin et. al. in *Headache*, **1988**, 28, 254-57 ; S. D. Silberstein et. al. in *Neurology*, **1992**, 42 (Suppl. 2), 6-10, P. J. Goadsby et. al. in *Ann. Neurol.*, **1991**, 29, 91-94 and C. Weiller et. al. in *Nature Med*, **1995**, 1, 658-60, and

c. the role of serotenin in pathogenesis of migraine as proposed by S. D. Silberstein in *Headache*, **1994**, 34, 408-17 and M. A. Moskowitz in *Ann. Neurol.*, **1984**, 16, 157-68.

However, many of these theories/hypotheses floating around for some time are being refuted or challenged.

Anti migraine therapy essentially consists of acute/abortive and prophylactic components.

In the recent past, several novel approaches to the treatment and prevention of migraine have been advanced. A wide array of drugs are available today to treat and prevent migraine, after the successful introduction of ergotamine tartarate and dihydroergotamine [Quality Standards Sub-Committee of American Academy of Neurology, *Neurology*, **1995**, 45, 585-87 and B. T. Hortone et. al., *Mayo. Clin. Proc.*, **1945**, 20, 241-48].

The last decade has witnessed a tremendous progress in acute abortive therapy of migraine using a new class of drugs, viz. the "triptans", which are prototypes of the Serotonin 5-HT_1 agonists. The "triptans", primarily acting via 5-$HT_{1B/D}$ receptor mechanism, can be administrated, nasally and orally and are found to be quick in action and generally provide 70% relief to migraine attacks in one hour compared to placebo (less than 27 %). However, some of the "triptans" exhibit certain pharmacodynamic and pharmacokinetic disadvantages, which limits their use for effective pharmacotherapy of migraine.

The number of agents for prophylactic treatment of migraine compared to that available for the abortive treatment are not large. The existing agents for the prophylactic therapy include, but are not limted to

i. β-blockers, such as propanol, metaprolol, nadolol, atenolol, and timolol which are effective in decreasing the frequency of attack [P. Stensrud et. al., *Headache*, **1980**, 20, 204-07 ; P. Kangasniemi et. al., *Cephalgia*, **1984**, 4, 91-96 ; R. E. Ryan Sr., et. al., *Headache*, **1983**, 23, 26-31 ; S. Diamond et. al., *Headache*, **1976**, 16, 24-27 ; J. W. Nadelman et. al., *Headache*, **1986**, 26, 175-82]. It is not clear whether their role in achieving prophylaxis is through catecholaminergic system or through 5-HT_2 receptors.

ii. Ca^{2+} channel antagonists, such as flunarizine and verapamil, which bring about a reduction in the frequency of attack

iii. Serotenin 5-HT_2 antagonists such as methysergide and pizotyline. The former is particularly effective in cases where the attack is severe, have high recurrence and do not respond to other medication [D. A. Curran et. al., *J. Neurol. Neurosurg. Psychiatry*, **1964**, 27, 463-69 ; E. Pederson et. al., *Clin. Pharmacol. Ther.*, **1966**, 4, 520-26 ; K. M. A. Welch, *New. Engl. J. Med.*, **1993**, 329, 1476-83]

iv. Tricyclic antidepressants, like amytryptiline and notryptiline given when the attack is aggrevated by tension, depression and insomnia.

v. Monoamino oxidase inhibitors, like phenelzine and isocarboxazid, given in cases where the headaches are refractory to standard treatment. These drugs are believed to have the ability to increase the levels of endogenous 5-HT and thereby useful in migraine prophylaxis [*Goodman and Gilman* "Pharmaçological Basis of Therapeutics", 9th Int. Ed., McGraw-Hill health professions Division, New York, **1996**, pp 486-89].

v. Anti-epileptic drugs such as sodium valproate, valproic acid and divalprox, effective in cases where the migraine attacks are associated with seizures, mania or anxiety [R. Jensen et. al., *Neurology*, **1994**, 44, 647-65 and N. T. Mathew et. al., *Arch. Neurol.*, **1995**, 52, 281-86].

However, in addition to several side-effects and shortcomings such as constipation, rebound headache, lethargy, depression, impotence, loss of hair, nausea, muscle cramps, aching, claudication, weight gain, hallucinations idiosyncratic retroperitioneal fibrosis, drowsiness, drying of mouth, blurred vision, urinary retension, cardiac arrhythmia, orthostatic hypotension, hepatotoxicity, alopecia, tremor etc. the rationale for administration and use of the abovementioned drugs is still not very clear.

The abovementioned shortcomings and non-availability of selective prophylactic therapeutic agents have led to the search for newer effective anti-migraine agents for the prophylactic and abortive therapy with less side effects and less toxicity profile.

New targets are being investigated for the prophylactic therapy of migraine and epilepsy, which share several clinical features and in many instances, respond to the same pharmacological agent. This suggests that similar mechanism(s) may be involved in their respective pathophysiology.

Amongst these, anticonvulsants as a class of drugs hold promise for migraine prophylaxis. These drugs are thought to act thorough multiple mechanisms involving voltage gated ion channels, ligand gated ion channels, GABA (γ-Amino Butyric Acid), Glutamate, Glycine, combined voltage/ligand gated ion channels and NMDA (N-Methyl D-Aspartate) [F. M. Cutrer, *Headache*, **2001**; (suppl) 1, s3-s10].

In the central nervous system, GABA is a major inhibitory neurotransmitter and known anticonvulsant drugs like sodium valproate and gabapentine have been shown to be effective in preventing migraine through modulation of GABA neurotransmission [R. Hering et. al., *Cephalalgia*, **1992**, 12(2), 81-84 and L. Magnus, *Epilepsia*, **1999**, 40(suppl 6), S66 – S72]. Others like Carbamazepine, used for treatment of trigeminal neuralgia has also been shown to be effective in the prophylaxis of migraine, primarily mediated by sodium

channels. Lomotrigine, a glutamate antagonist that blocks voltage gated sodium channels has also been demonstrated to be effective in migraine prophylaxis with aura [C. Lampl C et. al., *Cephalalgia*, **1999**, 19(1): 58-63]. Further, topiramate whose mechanism of action includes inhibition of voltage dependent sodium and calcium channels, AMPA (α-Amino-3-Hydroxy–5-Methyl-4-Isoxazolepropionic Acid)/Kainate glutamate receptors as well as enhancement of GABA-A receptor action is under extensive invention as a prophylactic agent for migraine [F. M. Cutrer, *Headache*, **2001**, (suppl) 1, s3-s10].

It might be mentioned here that all the anticonvulsants tested/under testing for the prophylaxis of migraine involve administration of the drug through routes other than nasal and their mechanism of action is not very clear.

The present invention identifies and characterizes new prophylactic targets for the antimigraine activity exhibited by the herbal extract prepared from *Sapindus trifoliatus*, the pharmacological activity of which was evaluated by intranasal route of administration. The aqueous of dried pericarp of *Sapindus trifoliatus*, containing a mixture of triterpenoid saponins was used for evaluation of the aforesaid activity.

Nasal sprays or drops are known for quick relief of migraine headaches. For example, nasal sprays/drops containing dihydroergotamine, sumatriptan succinate and lidocaine have been reported and used commercially.

OBJECT OF THE INVENTION

The principal objective of the present invention is to provide an aqueous extract, containing a mixture of triterpenoid saponins derived from the pericarp of fruits of the plant species *Sapindus trifoliatus*, possessing useful pharmacological activity.

An aspect of the present invention is to provide an aqueous extract, containing a mixture of triterpenoid saponins derived from the pericarp of fruits of the plant species *Sapindus trifoliatus*, possessing anticonvulsant activity, which is new and not reported hitherto so far.

A specific objective of the present invention is to provide an aqueous extract, containing a mixture of triterpenoid saponins derived from the pericarp of fruits of the plant species *Sapindus trifoliatus* useful for the prophylactic treatment of migraine.

Another specific objective of the present invention is to provide an aqueous extract, containing a mixture of triterpenoid saponins derived from the pericarp of fruits of the plant species *Sapindus trifoliatus* for the prophylactic treatment of migraine, mediated through its anticonvulsant activity.

Yet another objective of the present invention is to provide an aqueous extract, containing a mixture of triterpenoid saponins derived from pericarp of *Sapindus trifoliatus* wherein the said extract is highly effective for human use and capable for being used for the prophylactic treatment, relief and remedy of migraine.

Another objective of the present invention is to provide a process for the preparation of the aqueous, containing a mixture of triterpenoid saponins from the pericarp of *Sapindus trifoliatus*.

Further objective of the present invention is to evaluate the aqueous extract, containing a mixture of triterpenoid saponins derived from *Sapindus trifoliatus* for its *in vitro* receptor binding affinity towards the selected receptors, which have mediatory role in anticonvulsant activity.

Another further objective of the present invention is to evaluate *in vivo* the anticonvulsant activity of the aqueous extract, containing a mixture of triterpenoid saponins derived from *Sapindus trifoliatus* by intra nasal administration in rat of Maximal Electroshock Seizure (MES) test model.

Yet further objective of the present invention is to evaluate *in vivo* the anticonvulsant activity of the aqueous extract, containing a mixture of triterpenoid saponins derived from *Sapindus trifoliatus* in pentylene tetrazole (PTZ) seizure test model of rat by intra nasal administration.

Yet another further objective of the present invention is to evaluate the aqueous extract, containing a mixture of triterpenoid saponins derived from *Sapindus trifoliatus* for its effect on motor co-ordination in rats by intra nasal administaration in Rotarod performance test.

Other objective of the present invention is to find out the acute lethality dose (LD_{50}) of the aqueous extract, containing a mixture of triterpenoid saponins derived from *Sapindus trifoliatus* in mice and rats by intra nasal, intravenous and oral routes of administration.

A final objective of the present invention is to provide a pharmaceutical composition containing a pharmaceutically effective amount of the aqueous extract, containing a mixture of triterpenoid saponins derived from *Sapindus trifoliatus* useful in the treatment of certain indications.

Yet another final objective of the present invention is to provide a pharmaceutical composition containing a pharmaceutically effective amount of the aqueous extract, containing a mixture of triterpenoid saponins derived from *Sapindus trifoliatus* useful in the prophylactic treatment of migraine.

SUMMARY OF THE INVENTION

In accordance, the present invention provides an aqueous herbal extract, containing a mixture of triterpenoid saponins derived from *Sapindus trifoliatus*, which exhibits anticonvulsant activity, which is new and not reported hitherto so far. The anticionvulsant activity exhibited by the extract is particularly useful for the prophylactic treatment of migraine. The extract shows receptor binding affinity towards GABA-A agonist site, Glutamate-AMPA site, Glutamate-Kainate site, Glutamate-NMDA agonistic site, Glutamate-NMDA glycine (strychnine insensitive) site and Sodium channel (site 2), which is also new and hitherto not known.

DETAILED DESCRIPTION OF THE INVENTION

Sapindus trifoliatus, known as Ritha or Aristha belongs to the family of *Sapindaceae*. The fruit of the plant is used therapeutically as a tonic, purgative, emetic and expectorant [M. K. Nadkarni, *The Indian Materia Medica*, Vol I, 2nd Ed., Bombay Popular Prakashan, Bombay, India, **1982**, pp 1102-03]. It also possesses anti-inflammatory and analgesic actions [*Pharmaceutical Investigations of Certain Medicinal Plants and Compound Formulations used in Ayurveda and Siddha*, CCRAS, New Delhi, India, **1996**, pp 22-25]. It is also used as a spermicidal, in treatment of piles, hysteria, epilepsy and anti-implantation.

The pericarp of the fruit of the plant, which constitutes 62% of the fruit contains, glucose, saponins and primary metabolites. The saponins present in the fruit on acidic hydrolysis give the triterpenoid hederagenin, D-glucose, L-rhamnose and D-xylose [*The Wealth of India*, Vol IX, CSIR Publication, New Delhi, India, **1998**, pp 227-29]

Sapindus trifoliatus is pungent and bitter in taste. It has emetic actions i. e. it causes vomiting and nausea and is known to cause irritation of gastric mucosa, when administrated orally [P. V. Sharma, Dravyagunavignan (Hindi Commentary), VIIIth Ed., 1986, pp 384-86; *Pharmaceutical Investigations of certain Medicinal Plants and Compound Formulations used in Ayurveda and Siddha*, CCRAS, New Delhi, India, **1996**, pp 22-25].

Administration of *Sapindus trifoliatus* through nasal route is indicated for treatment of hemicarnia [M. K. Nadkarni, *The Indian Materia Medica*, Vol I, 2nd Ed., Bombay Popular Prakashan, Bombay, India, **1982**, pp 1102-03]. The therapy generally practiced consists of preparing an aqueous solution of the active ingredient and administration of the same through nasal route. However, there is no suggestion from the prior art about the effective concentration of the active ingredient, the preferred dosage required and duration of treatment. Moreover, it is not clear whether it is used as a curative or prophylactic and most importantly, its mechanism of action.

The pericarp was collected from local suppliers. It was identified by National Botanical Research Indstitute, Lucknow, India. The active ingredient i. e. pericarp of the fruit of

Sapindus trifoliatus can be used in the coarse form as such or it can be pulverized before use.

The pericarp can be extracted by percolation with water at room temperature for 16 hours or by boiling it with water. Similarly, the pericarp can be extracted by percolation with an alcohol at room temperature for 16 hours or by boiling it with an alcohol. The suitable alcohols are selected from ethanol, n-propanol, iso-propanol, n-butanol, iso-butanol and tert-butanol. Both water and the alcohol extracts show the presence of the principal saponins and other primary metabolites as evidenced by TLC and HPLC.

The water extract can be used as such or preferably is lyophilized and the lyophilized material thus obtained is reconstituted with appropriate quantity of water to achieve the desired concentration before use. Similarly, from the alcohol extract the solvent is evaporated to dryness under reduced pressure and further reconstituted with appropriate quantity of water achieve the desired concentration before use.

The saponins present in the aqueous/alcoholic extract have been identified and characterized as the following, viz.

	Compound Name	**Molecular weight**
Compound 1	β-D-Xyl -(1→ 2)- β-D-Ara-3-acetate -(1→ 3)- α-L-Rh -Hederagenin	924
Compound 2	β-D-Ara-4-acetate -(1→ 3)- α-L-Rh - (1→ 2)- β-D-Xyl - Hederagenin	924
Compound 3	β-D-Xyl-3,4-diacetate(1→ 3)- β-D-Xyl-4-Acetate-(1→ 4)-α-L-Rh-Hederagenin	966
Compound 4	β-D-Xyl- (1→ 3)- β-D-Xyl-4-Acetate-(1→ 4)- α-L-Rh-Hederagenin	924
Compound 5	β-D-Xyl (1→ 3)- α-L-Rh-(1→ 2) β-D-Xyl-3-Acetate-Hederagenin	924
Compound 6	β-D-Xyl (1→ 2)- α-L-Rh-(1→ 3) β-D-Xyl-Hederagenin	882

Acid hydrolysis of the extract yielded only one glycone, which was identified as hederagenin. Therefore, estimation of the abovementioned saponins present in the aqueous/alcoholic extract was calculated as hederagenin. From the estimation the concentration of hederagenin was found to be between 5.61-6.97 % by weight of the extract.

The aforementioned herbal extract exhibits receptor binding affinity towards GABA-A agonist site, Glutamate-AMPA site, Glutamate-Kainate site, Glutamate-NMDA agonistic site, Glutamate-NMDA glycine (strychnine insensitive) site and Sodium channel (site 2), As mentioned hereibearlier, the receptor binding affinity exhibited by the aforesaid extract is new and hitherto not known and which constitutes an important aspect of this invention.

The extract is useful in treatment of certain indications, such as hysteria, epilepsy, pain, asthma etc, in particular the prophylactic treatment of migraine. The receptor binding activity exhibited by the extract is useful in anticonvulsant activity. This anticonvulsant activity is believed to be useful in the prophylactic treatment of migraine.

in vitro receptor binding studies reveal that the extract of *Sapindus trifoliatus* exhibits binding affinity towards the receptor sites, which have a major mediatory role in its anticonvulsant activity .

The selected receptor binding affinity studies with the extract of *Sapindus trifoliatus* were conducted at NOVASCREEN®, USA for $GABA_A$ agonist site, Glutamate-AMPA site, Glutamate-Kainate site, Glutamate-NMDA agonistic site, Glutamate-NMDA glycine (strychnine insensitive) site and Sodium channel (site 2).

The results obtained on the above studies using the extract of *Sapindus trifoliatus* is summarized below.

Receptor	Receptor Source	Ligand	% Inhibition	
			2.5 µg/mL*	250 µg/mL*
GABA A, Agonistic site	Bovine Cerebellum	[^{3}H]GABA	50.92	102.40
Glutamate, AMPA site	Rat Forebrain	[^{3}H]AMPA	5.43	87.36
Glutamate, Kainate site	Rat Forebrain	[^{3}H]Kainic acid	-15.70	87.29
Glutamate, NMDA agonist site	Rat Forebrain	[^{3}H]CGP 39653	7.27	98.14
Glutamate, NMDA, Glycine (Strychnine-insensitive site)	Rat Cortex + Hippocampus	[^{3}H]-MDL-105,519	14.50	85.33
GABA, Chloride, TBOB	Rat Cortex	[^{3}H]TBOB	-5.12	85.03
Glutamate, Chloride	Rat Cerebellum	[^{3}H]Glutamic Acid	-2.72	89.49
Sodium, Site 2	Rat Forebrain	[^{3}H] Batrachotoxin A 20-a-Benzo	19.98	69.54

(*) Refers to the lyophilized powder obtained from the aqueous extract of *Sapindus trifoliatus*

Further, the extract of *Sapindus trifoliatus* exhibited dose dependent binding affinity to $GABA_A$ agonistic site, with IC_{50} value of 1.74 µg/ml (Ki 1.70 µg/ml). The extract of *Sapindus trifoliatus* also exhibited dose dependent binding affinity to glutamate-NMDA agonistic site with IC_{50} 0f 140 µg/ml (Ki 113µg/ml).

The IC_{50}/Ki determination study for $GABA_A$ agonist site and Glutamate-NMDA indicate that the extract has dose dependent binding affinity to $GABA_A$ agonistic site and glutamate-NMDA agonistic site.

From *in vivo* studies it is observed that the extract prevented the hind limb extensor phase in rats, which moreover, is dose dependent in a Maximal Electroshock Seizure (MES) model. This clearly indicates prevention of seizure spread on intranasal administration.

Description of the method of evaluation for anticonvulsant activity in MES model

The nature of the binding affinity of the extract of *Sapindus trifoiliatus* was further investigated in functional assays using *in vivo* animal models.

In order to evaluate the efficacy of the extract of *Sapindus trifoiliatus*, for its prophylactic therapeutic potential in migraine, its role as an anticonvulsant was evaluated in an *in vivo* animal model. Maximal Electroshock Seizure (MES) [E. Swinyard et. al., *J. Pharmacol Exp Ther.*, **1952**, 106: 319-330] test model was employed for the efficacy evaluation. Drugs acting on the receptors like Glutamate–NMDA,Glutamate–AMPA /Kainate,Glycine site and voltage dependent Na^+ channels are known to inhibit MES induced seizures (S. S. Lin et. al., SS., *CNS Drug Reviews*, **1999**; 5(4): 365-78 and H. S. White et. al., *Ita.J.Neurol Sci.*, **1995**; 16(1-2): 73-7]. Male Wistar rats (150 – 200 g) were used in the study. *Sapindus .trifoiliatus*, dissolved in saline was administered intranasally in a volume of 250µl/kg in a dose range of 0.25mg/kg to 25mg/kg. After administration of either the test compound or an equivalent volume of the vehicle (for control experiments) or standard drug, the rats were observed for any tremors or convulsions. Thirty minutes after intranasal administration the rats were administered electroshock (100Hz, 150mA, 0.2 sec) by bipolar pinna electrodes using an electroconvulsometer (INCO, India). The incidence, latency as well as duration of hind limb extension were noted. Mortality if any was recorded. Abolition of the hind limb tonic extensor component indicates the test compound's ability to inhibit MES-induced seizure spread.

The extract of *Sapindus trifoiliatus* administered intranasally at a dose range of 2.5mg/kg to 25mg/kg in a volume of 250µl/kg abolished the hind limb tonic extensor phase in the MES induced seizures in rats. The ED_{50} for the extract of *Sapindus trifoiliatus* was determined to be 7.72 mg/kg, i.n., while that of Sodium valproate was 67.70 mg/kg, i.p., as summarized below. ED_{50} represents protection to hind limb tonic extension due to electroshock.

Treatment*	ED_{50} values	95% confidence limits
S.trifoilatus	7.72 mg/kg, i.n.	5.28 to 11.04 mg/kg i.n.
Sodium valproate	67.67 mg/kg, i.p.	53.53 to 80.30 mg/kg i.p.

(*) No. of animals at each treatment level 5–10.

The extract of *Sapindus trifoliatus* did not cause protection against PTZ induced convulsions in rats on intra-nasal administration.

Description of the method of evaluation for anticonvulsant activity in PTZ model

Male Wistar rats (150 – 200 g) were used in the study. The extract of *Sapindus trifoiliatus* dissolved in saline was administered intranasally at two high concentrations 250 mg/kg and 375 mg/kg in a volume of 250µl/kg based on solubility and syringability for instillation into the nasal cavity. After administration of either the test compound or an equivalent volume of the vehicle (for control experiments) or standard drug, the rats were observed for any tremors or convulsions. Fifteen minutes after intranasal administration rats were administered pentylenetetrazole (60 mg/kg,i.p. 2 ml/kg) and the incidence and latency of myoclonic jerks as well as generalized seizures were noted for a period of 30 minutes. Also severity was ranked on a scale of 0-5. Mortality if any were recorded. Severity was ranked as follows: Stage 0 –No response, Stage 1 – Ear and facial twitching, Stage 2- Myoclonic jerks without upright posture, Stage 3 – Myoclonic jerks, upright position with bilateral forelimb clonus, Stage 4- Clonic tonic seizures, Stage 5 – Generalized clonic – tonic seizures, loss of postural control. Diazepam (4mg/kg,i.p., 2ml/kg) was used as the standard control. Absence of generalized clonic convulsions of stage 5 severity indicates compound's ability to be protective in nature.

The extract of *Sapindus trifoliatus* administered intranasally at two high concentrations 250mg/kg and 375 mg/kg in a volume of 250µl/kg did not afford protection of PTZ induced seizures in rats. However diazepam (4mg/kg,i.p., 2ml/kg) used as the standard control significantly protected the seizures induced due to PTZ. The rats did not show any tremors or convulsions due to *Sapindus trifoliatus* treatment prior to PTZ administration.

The extract of *Sapindus trifoliatus* did not effect motor co-ordination in rats on intra nasal administration indicating lack of neurological impairment at the doses studied.

Description of the method of evaluation of motor co-ordination on rota rod performance tests in rats

Drugs with anticonvulsant activity that do not exhibit sedation or death in animal models are considered safe. Hence the effect of *Sapindus trifoliatus* was evaluated for the same on rota rod performance test in rats.

Wistar male rats (150 – 200g) pre-trained, were subjected to rotarod (Letica, Spain) test (15 rpm) for sixty seconds at intervals of 0, 5, 10, 15, 20, 30 and 45 minutes post intranasal treatment of test compound or an equivalent volume of the vehicle (M. S. Dunham et. al., *J. Amer. Pharmac. Assoc. Sci. Edit.*, 1957, 46, 208-209). The extract of *Sapindus trifoliatus* dissolved in saline was administered intranasally at two high concentrations 250 mg/kg and 375 mg/kg in a volume of 250µl/kg based on solubility and syringability for instillation into the nasal cavity. The inability to balance for sixty seconds was considered as lack of motor in-coordination by the compound. Diazepam (4mg/kg,i.p., 2ml/kg) was used as the standard control.

At the doses of 250 mg/kg and 375 mg/kg in a volume of 250µl/kg administered intranasally to rats , the extract of *Sapindus trifoliatus* did not affect motor co-ordination upto 45 minutes post treatment in rotarod performance test.

Further Studies suggest that the extract which shows affinity towards receptors that have a mediatory role in anticonvulsant activity, however, does not induce or potentiate convulsions of chemical or electrical origin.

Preclinical pharmacological data from receptor binding and *in vivo* studies clearly indicate anticonvulsant activity of the extract. The anticonvulsant activity has been demonstrated in the MES model by the intra nasal route of administration without sedation.

The toxicological studies for acute lethality dose (LD_{50}) of the extract of *Sapindus trifoliatus* were conducted in both mice and rat by using intra nasal route. Further, to find out lethal

dose by other routes (both intravenous and oral) were also employed. Mice and rats were observed for a period of 14 days after treatment with the extract.

The acute lethality dose (LD_{50},mg/kg) of the extract of *Sapindus trifoliatus* was found to be >270 (intranasal), >1250 (oral) and >150 (intravenous) in mice while in rats it was found to be >90 (intranasal), > 1000 (oral) and >80 (intravenous).

The extract of *Sapindus trifoliatus* is further found to be safe in safety pharmacological studies.

Batches of nasal spray containing the lyophilized aqueous extract of *Sapindus trifoliatus* equivalent to 0.004, 0.013, 0.027 and 0.08 % of hederagenin by weight of the aqueous extract have been formulated in combination suitable pharmaceutically acceptable carriers or vehicles.

The invention is further illustrated by the following non-limiting examples.

EXAMPLE 1

Extraction of the pericarp of Sapindus trifoliatus with water

Dry pericarp of *Sapindus trifoliatus* obtained from local suppliers was used as the starting material. The raw material 100g was soaked in 400ml of Milli-Q water and left for 16 hrs. The percolate was then decanted, centrifuged and filtered through Whatman filter paper (No.1) to give a clear extract (300ml). The process of extraction was repeated three times with same volume of solvent. The percolate obtained in the second and third percolations were 400ml each. These were pooled and lyophilized to give a brown coloured powder in a yield was 68%.

EXAMPLE 2

Extraction of the pericarp of Sapindus trifoliatus with n-butanol

The dry pericarp of *S. trifoliatus* (50.05g) was soaked in 250ml of n-butanol and left for 16 hrs. The percolate was then decanted, centrifuged and filtered through Whatman filter paper (No. 1) to give a clear extract (208ml). The process of extraction was repeated three times with same volume of solvent. The percolate obtained in the second and third percolations were 244 and 250ml, each. These were pooled and lyophilized to give a brown coloured powder, in a yield of 13.51%

EXAMPLE 3

Extraction of the pericarp of Sapindus trifoliatus with iso-propanol

The dry pericarp of *S. trifoliatus* (50.06g) was soaked in 250ml of iso-propyl alcohol (IPA) and left for 16 hrs. The percolate was then decanted, centrifuged and filtered through Whatman filter paper (No.1) to give a clear extract (205ml). The process of extraction was repeated three times with same volume of solvent. The percolate obtained in the second and third percolations were 240 and 246ml, each. These were pooled and lyophilized to give a brown coloured powder in a yield of 15.4%.

Dated this 27th day of August 2002.

S. MAJUMDAR
of S. MAJUMDAR & CO.
Applicants' Agent